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Does COPI Go Both Ways?

Minireview

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The classic model of protein transport within the eukaryotic cell predicts a role for vesicles that ferry cargo proteins along well-defined routes of membrane traffic (Figure 1). Clathrin-coated vesicles serve as a paradigm for this mechanism. Clathrin adaptor proteins bind to cytoplasmic domains of receptor proteins, and the clathrin triskelion nucleates the assembly of a cage that concentrates receptors and their ligand cargo into a bud. Rearrangement of the coat subunits pinches off a bit of plasma membrane carrying molecules destined for transport to endosomes, leaving behind those molecules designed to remain and function at the cell surface.

Protein traffic early in the secretory pathway does not involve clathrin. In 1986, Orci et al. reported the existence of a novel, nonclathrin coat that envelops buds and vesicles that form from Golgi membranes engaged in the transport of a viral glycoprotein, vesicular stomatitis virus G protein (VSV G) (Orci et al., 1986, and Figure 2). This discovery led to the characterization of a complex, called coatomer, responsible for the formation of COPI-coated vesicles. A great deal of effort in the past 10 years has been devoted to the biochemical, morphological, and genetic analysis of coatomer function. Until now, however, no direct connection between coatomer and anterograde protein traffic within an unperturbed, intact cell had been established. In a new report, Orci et al. (1997 [this issue of *Cell*]) suggest such a direct link with the observation of a population of Golgi-associated COPI vesicles that carry proinsulin in insulin-secreting cells of the pancreas. These results are particularly important in light of recent developments that indicate an alternative but not mutually exclusive role for COPI in the retrieval of proteins in opposition to the anterograde flow of traffic (Letourneur et al., 1994; Pelham, 1994).

Two Views of Coatomer Function

The original observations of Rothman and Orci were consistent with a role for COPI in anterograde traffic within the Golgi complex. VSV G protein was detected within COPI buds and vesicles in cell-free reactions in which G protein was transferred into compartments that successively mature the N-linked oligosaccharide moiety (Orci et al., 1989). Biochemical resolution of this system has shown that pure coatomer complex and the small GTPase, ARF, and nucleotide were sufficient to collect VSV G into buds and vesicles from isolated Golgi membranes (Ostermann et al., 1993). The COPI-coated vesicles were interpreted as being functional largely because their VSV G protein cargo was transferred in vitro from one Golgi complex to another under conditions that mirrored the conditions required for vesicle formation, docking, and fusion. However, to many investigators in the field, it remained uncertain whether the vesicles represented an obligatory intermediate in intra-Golgi transport in the in vitro reaction in intact cells.

The importance of COPI to anterograde transport in the secretory pathway was clearly supported by a variety of subsequent studies. First, the macrocyclic antibiotic brefeldin A (BFA), an inhibitor of guanine nucleotide exchange activity needed to promote ARF binding to membranes, was found to block transport efficiently and to result in a general disassembly of the Golgi complex (Lippincott-Schwartz et al., 1989). Likewise, certain mutations in genes encoding coatomer subunits (in both yeast and animal cells) as well as the microinjection of antibodies to the coatomer subunit β-COP blocked forward transport of both membrane and secretory proteins (Hosobuchi et al., 1992; Pepperkok et al., 1993). Thus, COPI components were required for anterograde transport either to or through the Golgi complex in intact cells. Independent support for the coatomer hypothesis came with the discovery that anterograde vesicular transport from the endoplasmic reticulum (ER) is executed by a similar coat, COPII, and a related GTPase, Sar1p (Barlowe et al., 1994; Aridor et al., 1995).

Given the importance and generality of the coated

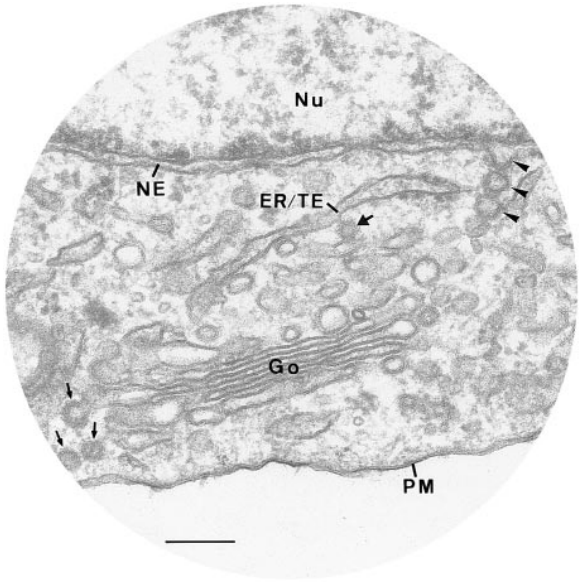

Figure 1. Typical Organizational Pattern of the Secretory Pathway in a Eukaryotic Cell, as Seen by Thin-Section Electron Microscopy

From the nuclear envelope (NE) and the ER, transport progresses through transitional elements of the ER (ER/TE) that face the *cis* pole of the Golgi complex (Go). The *trans* pole of the latter faces the plasma membrane (PM). Transport vesicle buds are seen from the nuclear envelope (arrowheads), from the transitional element of the ER (bold arrow), and from the lateral rim of Golgi cisternae (thin arrows). Nu, nucleus. Scale bar represents 200 nm. (Micrograph courtesy of Lelio Orci.)

vesicle hypothesis, the role of coatomer in the cell-free system and in intact cells has enjoyed more than the usual level of scrutiny. Numerous questions have been raised, some of which have been discussed previously (Mellman and Simons, 1992). The major new alternative, that coatomer serves an essential and possibly exclusive role in retrieval from the Golgi back to the ER, is particularly compelling.

COPI and retrograde transport first emerged as an attractive hypothesis with the observation that coatomer mutations in yeast block retrieval from the Golgi complex of resident ER membrane proteins (Letourneur et al., 1994). Retrieval of escaped ER proteins and recycling of vesicle targeting proteins (v-SNARES) is, of course, essential to the ongoing activities of a vesicular transport pathway. Thus, mutations that block retrograde transport would be expected to indirectly but inevitably shut down secretion. From this perspective, any inhibitor of coatomer function (e.g., BFA, antibodies, or lethal mutations) would block anterograde transport even if the effect is exclusively on retrieval (Gaynor and Emr, 1997). Indeed, the existence of COPII, whose only function seems to be anterograde vesicular transport of cargo from the ER to Golgi, could be interpreted to mean that COPI existed primarily, if not exclusively, for retrograde transport.

Anterograde Transport without Vesicles?

If COPI is utilized uniquely for retrograde transport, how then might anterograde transport across the Golgi stack be mediated? Thus far, two alternatives to COPI-mediated vesicle traffic have been suggested. The first alternative was prompted by the observation that VSV G transport in the cell-free system was insensitive to BFA and proceeded with normal efficiency even in the absence of ARF and therefore in the absence of assembled COPI complexes (Taylor et al., 1994). Since BFA induces a dramatic degree of tubule extension from cisternal membranes, the idea emerged that vesicle-independent forward transport might occur via regulated tubule formation, resulting in transient intercisternal connections. The gating of such tubules could account for unidirectional progression of cargo. Intercisternal connections and tubules can be observed even in the untreated Golgi, both by thin-section electron microscopy and by video microscopy of living cells. However, such connections are observed infrequently, relative to the number of buds and vesicles that congregate around the dilated cisternal rims of a Golgi stack. Tubules have even been reported to connect the ER and *cis* Golgi, and yet, at least at this stage in the pathway, there seems to be little dispute thus far that COPII vesicles mediate anterograde transport from the ER.

In the context of the cell-free system, it is easy to see that an imbalance in coatomer or ARF could lead to unprogrammed fusion between neighboring Golgi cisternae. Fusogenic proteins are probably regulated to promote only appropriate membrane encounters. Perhaps the fusogens become activated and then sequestered by a coated surface or bud. BFA or any of the coat inhibitors may create unnatural interactions that lead to illegitimate fusion events. A breach in the normal compartmental boundary would lead to intermixing of glycosyltransferases and the viral glycoprotein substrate, giving the appearance of a signal in the transport reaction independent of COPI vesicle formation.

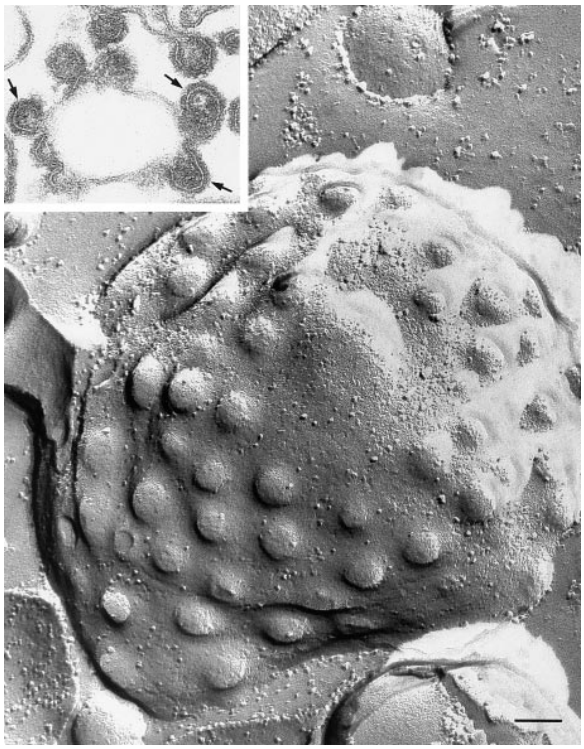

Figure 2. Freeze-Fracture View of an Isolated Golgi Cisternal Element Incubated in Conditions That Promote COPI Vesicular Budding

The membrane fracture face is deformed by numerous circular bulges, each of them representing the emergence of a coated bud. Inset: Thin-section view of a comparable cisternal element with coated buds (arrows). Scale bar represents 100 nm. (Micrograph courtesy of Lelio Orci.)

A second model of anterograde flow within the Golgi complex suggests that each cisterna is formed by the fusion of ER-derived transport vesicles and then matures by progressive migration in the *trans* direction (Schnepf, 1993). Each cisterna would acquire a new set of Golgi maturation enzymes (glycosyl trimming and transfer, sulfation, and proteolytic maturation) by COPI-mediated retrieval from a distal compartment. The size of the stack may be maintained by a balance in the consumption of *trans*-most and formation of *cis*-most cisternae. Chief among the virtues of this model are that no distinction need be made between anterograde and retrograde vesicles; no anterograde fusion is called for; and large particles, possibly exceeding the inclusion limit of a transport vesicle, would be swept along at the pace of cisternal migration.

Although the idea of cisternal progression and maturation first emerged in morphological inspection of traditional secretory systems, its clearest support has come from the analysis of cell wall scale production in several algal species (Melkonian et al., 1991). Scales are assembled within Golgi cisternae, typically first appearing near but not directly within this *cis*-most cisternae. The structures consist largely of polysaccharide (~90% by mass)

that seems to mature in the *trans* direction. Some scales are much too large to be accommodated within normal Golgi-associated transport vesicles; others (20–50 nm in diameter), however, clearly could but nevertheless seem not to be. Exocytosis of each completed scale occurs by direct fusion of a *trans* Golgi saccule to the plasma membrane. In its earliest formulation, the problem with this model was how to explain the regionalized localization of Golgi resident proteins necessary to mature the scales or typical secretory product. The possibility that COPI vesicles may act continually to retrieve resident Golgi enzymes of the *cis* and medial Golgi as they "progress" along with cisternae toward the *trans* face of the stack has made cisternal progression a popular model (Harris and Waters, 1996). It is one that is consistent with virtually all of the available genetic and biochemical evidence, the major problem being, however, that evidence for cisternal progression remains indirect.

Given the possibilities of tubular connections among Golgi cisterna, cisternal progression and maturation, and COPI vesicle–mediated recycling of resident Golgi proteins, many investigators have been willing to dismiss the original observations of anterograde VSV G transport within COPI vesicles as some kind of in vitro artifact.

Anterograde and Retrograde COPI Vesicles?

The work of Orci et al. (1997) provides a potential way out of the dilemma by reconciling the original idea that anterograde transport across the Golgi stack is mediated by COPI-coated vesicles with the more recent idea that COPI mediates retrograde traffic. Using both immunocytochemical and biochemical approaches, Orci et al. provide data suggesting that COPI may be involved in vesicular transport in both directions. Their work presents evidence for two distinct populations of Golgi-associated COPI vesicles in pancreatic β cells: one containing the secretory marker protein, proinsulin, and the other containing the KDEL retrieval receptor, Erd2. Although vesicles in cells do not come addressed with arrows, it seems likely that proinsulin marks a population of anterograde transport vesicles in transit along the Golgi stack and that Erd2 reflects the population of retrograde vesicles retrieving resident Golgi proteins to proximal cisternae. Additional data from the in vitro transport system suggest that two populations of COPI vesicles are formed, only one of which can be perturbed by the addition of bivalent antibody directed against the cytoplasmic tail of VSV G protein. The existence of two populations of COPI vesicles may in part explain the apparent coincidence of Golgi resident proteins and VSV G in enriched COPI vesicles described previously (Ostermann et al., 1993).

The data presented by Orci et al. (1997) are limited in some respects. For example, equal amounts of Erd2 must be included in the anterograde transport system in order for it to appear in retrograde vesicles, so a complete segregation of Erd2 from an anterograde marker might not have been expected a priori. We must also assume that the bulk of proinsulin proceeds unidirectionally across the stack toward forming secretory granules, where it is cleaved (yielding insulin) and thus no longer detected by the antibody used for these studies. In addition, there must be some difference, in sorting or other functional property, that would allow the same coat protein complex to form vesicles capable of moving in opposite directions. Nevertheless, Orci et al. have directly raised an attractive possibility: that COPI vesicles are responsible for bidirectional transport.

What do these new observations say about the alternative modes of anterograde transport? Although transport through tubules and transport by cisternal progression remain viable options, the justification for these views has always been based on negative evidence: the failure to detect transport intermediates in vesicles. One assumes with no direct evidence that large particles such as algal scales, rigid polymers of collagen, casein micelles, lipoproteins, or enveloped virus cannot be accommodated within the usual transport vesicle. If so, then how are we to explain the vesicular transport of their precursors from the ER, a process that most now agree is accomplished by COPII, the cousin of COPI?

Two examples illustrate the limitations of the size exclusion argument as it applies to the vesicular transport of large particles. Very low density lipoprotein particles (30–80 nm) made in the liver are assembled, in part, during translation and translocation of ApoB in the rough ER. Mature particles are first detected in the smooth ER in regions of transition to the Golgi (Claude, 1970). Large accumulations of very low density lipoprotein particles concentrate at the dilated rims of Golgi cisternae prior to their capture in mature secretory granules. This pattern of transport is recapitulated in the synthesis and transport of chylomicrons (l80–500 nm in diameter) within absorptive epithelial cells of the intestine (Sabesin and Frase, 1977). Although a detailed analysis of the transit of these large lipoproteins within the Golgi complex has not been conducted, it is clear that some form of traffic, almost certainly vesicular, mediates their transit from the ER to *cis* Golgi. In addition, large viral particles (herpes virus and vaccinia virus, with diameters of 120–600 nm) may either bud directly into the *trans* Golgi network or disrupt the cisternal organization of the stack (Schmelz et al., 1994). In any event, a size restriction would apply only if coat proteins responsible for budding were incapable of accommodating the geometry of a larger than normal particle. At least in the case of clathrin-mediated endocytosis, particles as large as herpes virus can be enclosed within coated pits and coated vesicles that are two times their normal diameter, and even larger for particles that induce phagocytosis. Therefore, the size limit argument may not be useful.

What Now?

The new immunocytochemical observations of Orci et al. (1997) raise the standard of analysis in this field. Is the case closed on vesicles versus tubules versus maturing cisternae? Probably not! The age distribution of investigators in this field ensures that many more years of effort will be devoted to discriminating the various contending models of protein transport.

At this juncture it seems worthwhile to raise several very specific questions that are within experimental grasp and that may help ultimately to solve the mechanism of transport across the Golgi.

First, providing direct and definitive proof in favor of the cisternal progression or the COPI vesicle–mediated

anterograde transport models will be difficult. As a gedanken experiment, the optimal test would be to accumulate a "pulse" of precipitated macromolecules or a large structure in the *cis*-most cisterna and then to monitor whether the structure traverses the stack as a function of chase time. An example would be to regulate the production of algal scales, allowing them to accumulate in only a single cisterna and then to turn off scale synthesis and assay by electron microscopy whether it progressed to medial and then to *trans* cisternae.

Second, what is the role of coat proteins in the secretion of large particles? Quantitative immunocytochemical analysis may be used to inspect the localization of COPI and COPII subunits on membranes engaged in the maturation of particles such as lipoproteins, algal scales, and polymers of collagen. COPII, which is involved exclusively in vesicular anterograde transport, will be required for transport of particles from the ER unless some other mode of traffic is possible. Lipoprotein-secreting cells transfected with a dominant mutant form of Sar1p, the GTPase employed to nucleate COPII assembly, may be used to test this possibility.

Third, are there Golgi SNARE molecules exclusively involved in anterograde transport? Only the tubule and vesicle hypotheses invoke specific fusion events in the transmission of cargo molecules from *cis* to *trans* in the Golgi complex. Among SNAREs predicted from the sequence of the yeast genome, one or more may be found within the Golgi complex and function in anterograde traffic. For example, will distinct populations of COPI vesicles contain anterograde- and retrograde-directed SNARE molecules?

Once completed, experiments such as we suggest may do nothing more than demonstrate that transport through the Golgi stack makes use of both anterograde vesicular traffic and cisternal progression, perhaps at two decidedly different rates. In any case, the field of protein transport will have progressed in the anterograde direction.

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